A Multiwavelength, Common-mode Architecture for a Digital Holographic Microscope

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22 May 2019
3190936
2019 OSA Digital Holography and 3D Imaging

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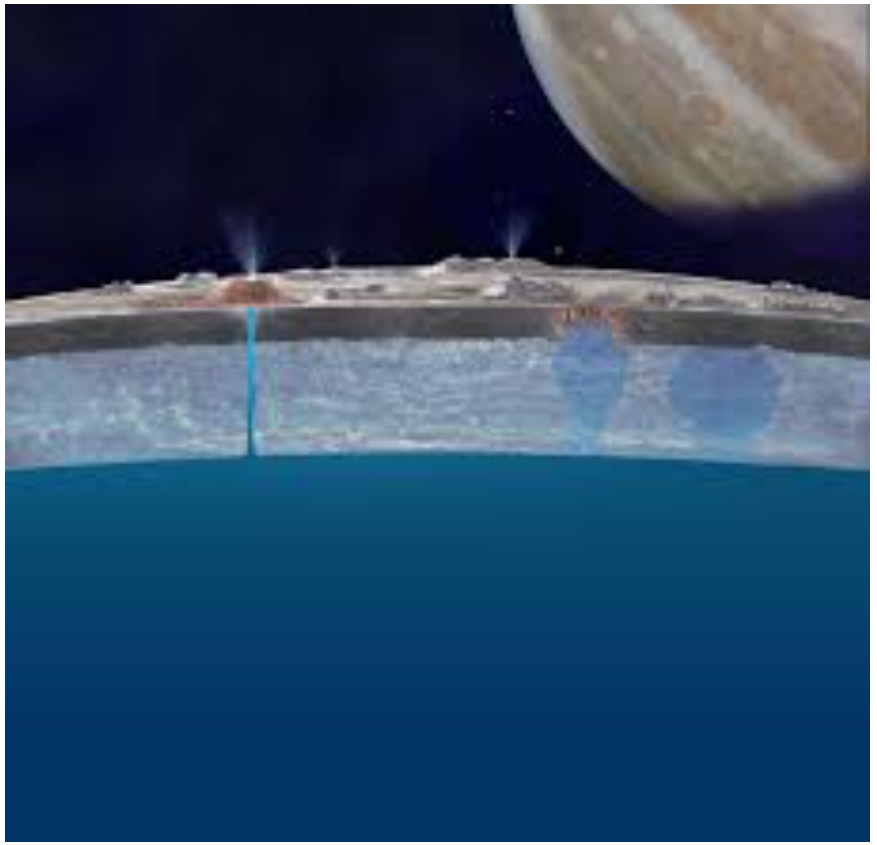
This work was supported in part by the Gordon and Betty Moore Foundation through Grant GBMF4038 to the California Institute of Technology, and by JPL's internal R&TD and JNEXT programs.

Searching for life on icy moons is increasingly compelling.

- These moons contain vast reservoirs of liquid water that appear to be driven by thermal sources.
- Extant life on these worlds is almost certainly microbial.
- A lander has been extensively studied and is feasible although its future is uncertain.



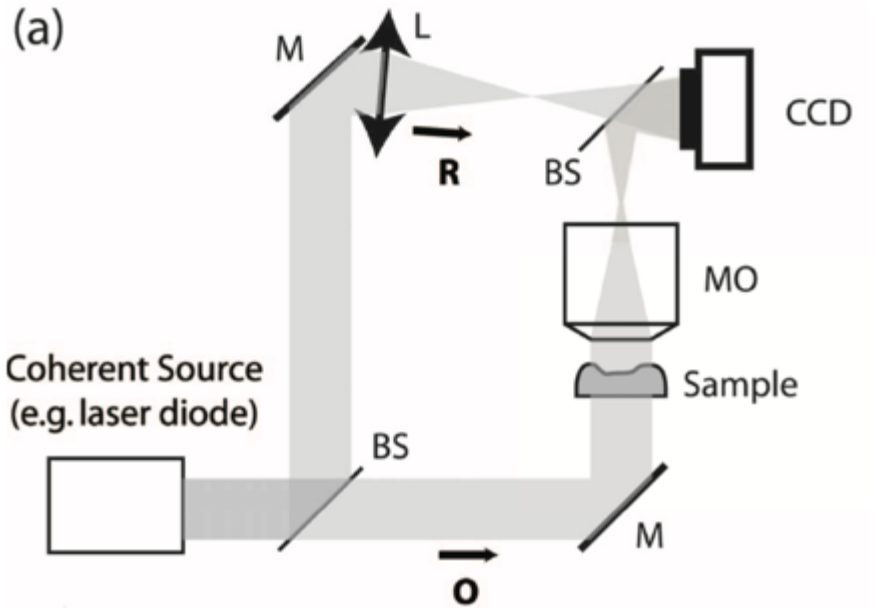
How do we tell if there is extant life?

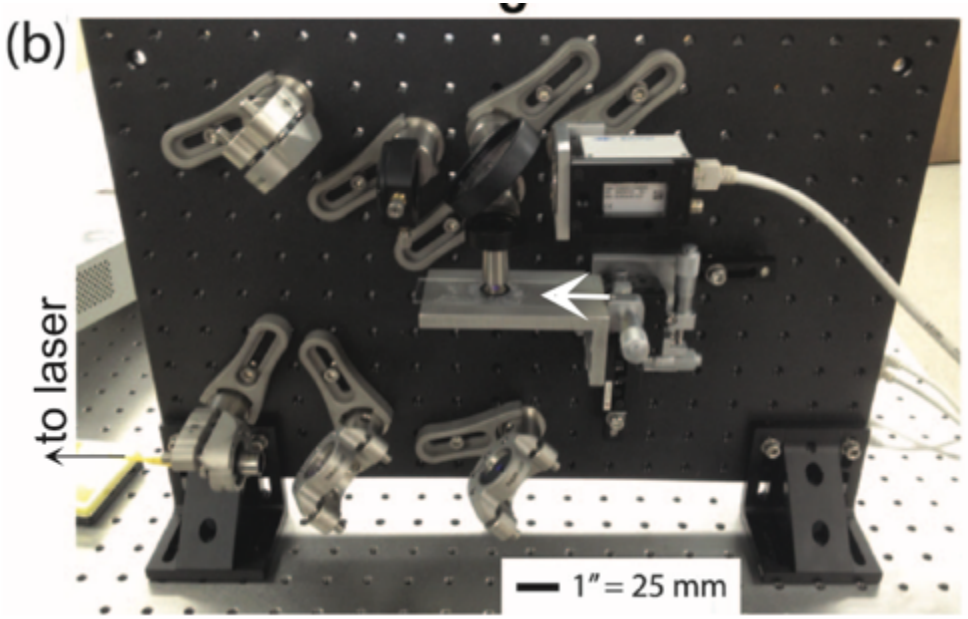
- How do we detect extant life?
  - DNA analysis may sound like the answer, but the answer may be inconclusive.
  - Viruses can be analyzed, but are they alive? They cannot metabolize or reproduce without a host
  - Chemical complexity as a criteria is actually not well defined.
- Microscopy is the gold standard in biology
  - What advantages does it afford?
  - What are the pitfalls?

Advantages of Microscopy

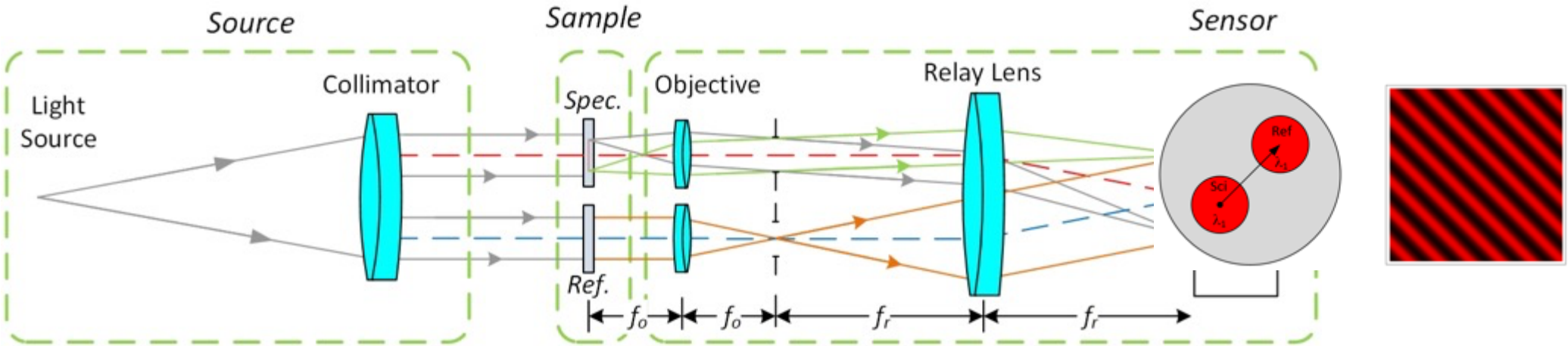
- Microscopic images provide a great deal of information about about the simplest cells
  - Structure
  - Physical Properties
  - Activity
- Holographic Microscopy has even more advantages.
  - It is essentially and optical interference technique.
  - It measures the full electric field (both amplitude and phase).
  - Has no moving parts.
  - Allows volumetric imaging.
  - And if engineered properly…

Classic Architecture for Digital Holography





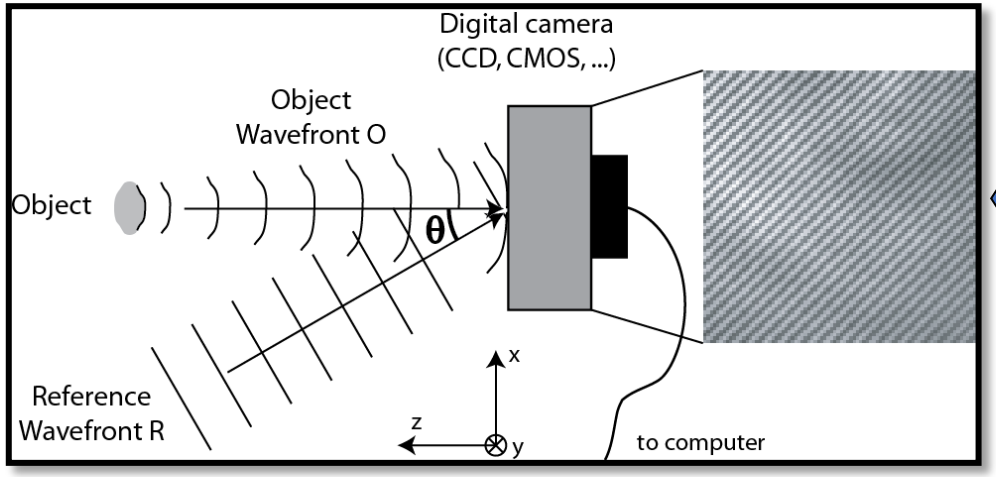
Digital Holographic Microscopy or DHM



<u>Wavelength</u>: 405 nm
<u>Objective NA</u>: 0.30
<u>Resolution</u>: ~ 0.8 µm*
<u>FOV</u>: 360 x 360 µm
<u>Depth of Focus</u>: ~ 1 mm

Wallace, J. Kent, et al. "A Robust, Common-Mode Digital Holographic Microscope." *Optics Express* 23(13): 17367-17378 (2015)

DHM Data Processing



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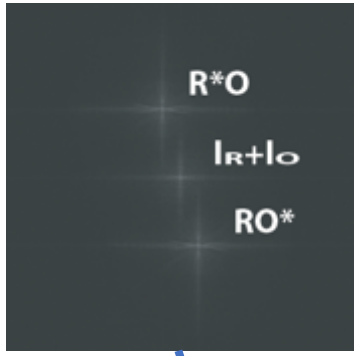
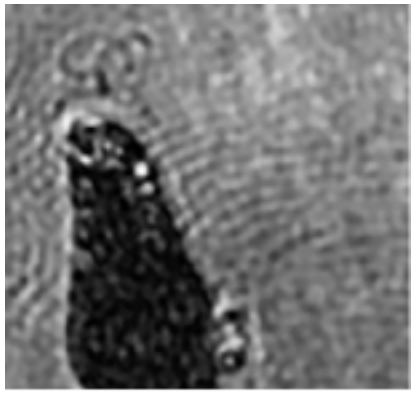
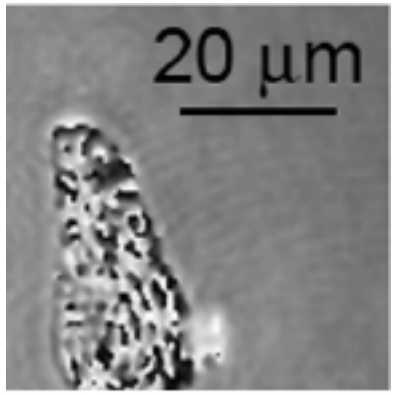


Intensity



$|\Gamma(\xi,\eta)|^2$

Phase



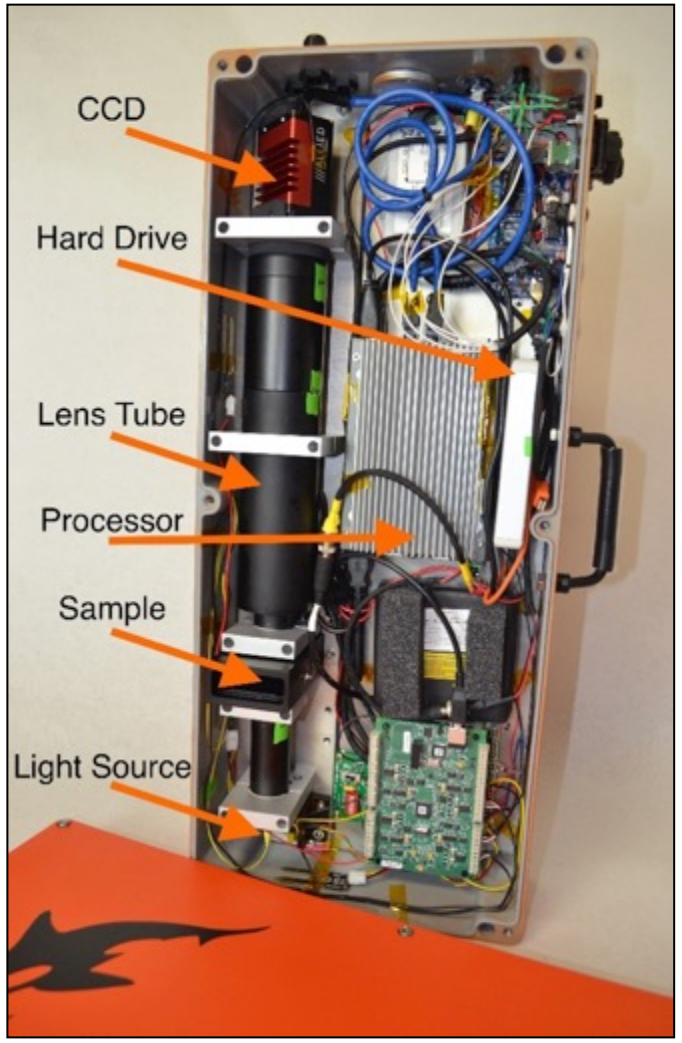
$\Gamma(\xi,\eta) = \Im^{-1}\{\Im(h \cdot R) \cdot G\}$

$\arctan(\mathrm{Im}[\Gamma(\xi,\eta)]/\mathrm{Re}[\Gamma(\xi,\eta)])$

- *The filtered hologram is reconstructed into an intensity image (equivalent to brightfield) and a quantitative phase image (not phase contrast) by using a propagator to calculate the field at the desired plane.*
- *Phase imaging allows for label-free imaging of transparent objects (many biological cells)*

First DHM Field Instrument

- All in one instrument including DHM, processor, hard drive, power supply, and diagnostic equipment
- Push button operation, WiFi, or hard wired connection to external computer
- Custom sample chamber cartridges for easy sample loading
- Fits inside standard passenger aircraft carry-on size limit



Lindensmith, Christian A., et al. "A submersible, off-axis holographic microscope for detection of microbial motility and morphology in aqueous and icy environments." *PloS one* 11.1 (2016): e0147700.

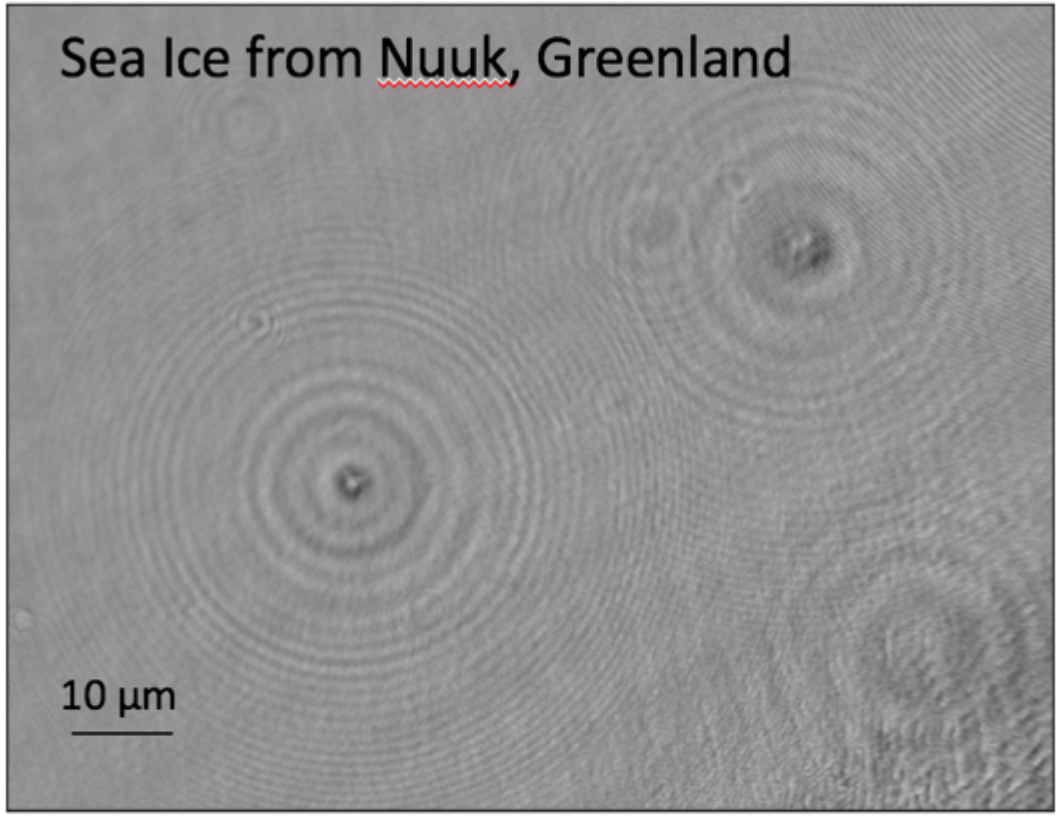
Lindensmith, Christian A., et al. "A submersible, off-axis holographic microscope for detection of microbial motility and morphology in aqueous and icy environments." *PloS one* 11.1 (2016): e0147700.

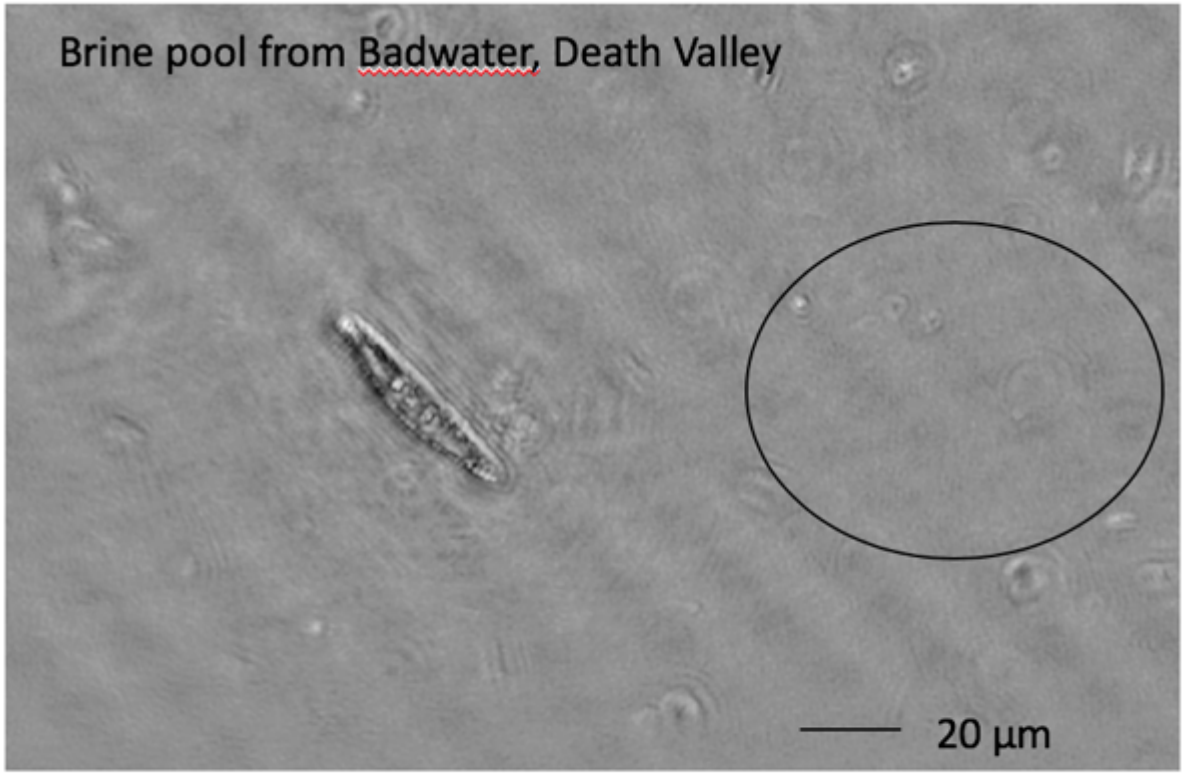
Deployments

Utqiaġvik (Barrow) Alaska, May 2017

Badwater,
Death Valley, CA

Motility as a sign of life: results from real field environments taken *in situ*

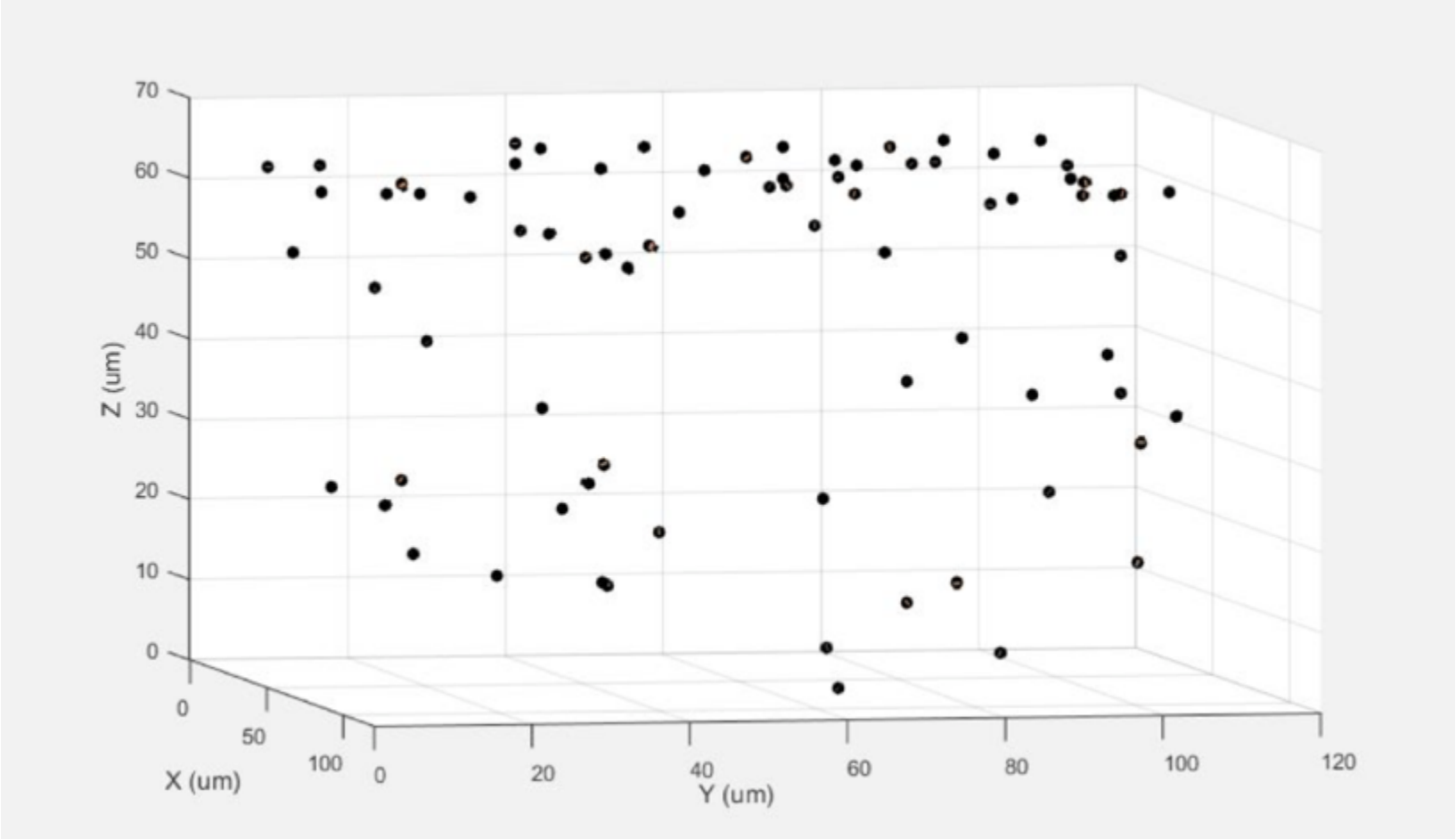




Circle indicates region traversed by a prokaryote during video

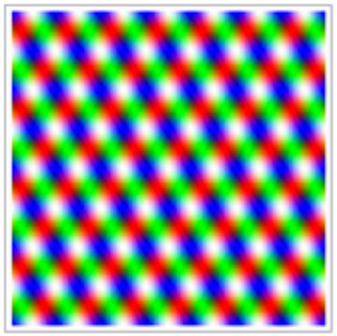
Hypothesis: **active microbes are an inherent feature of natural aquatic habitats, even extreme (subzero) ones**. Not all inhabitants may rely upon movement to complete their life histories, but some fraction of the community will have evolved the ability to achieve directed motion via swimming or gliding

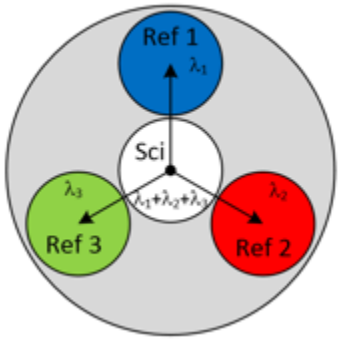
3D Tracking

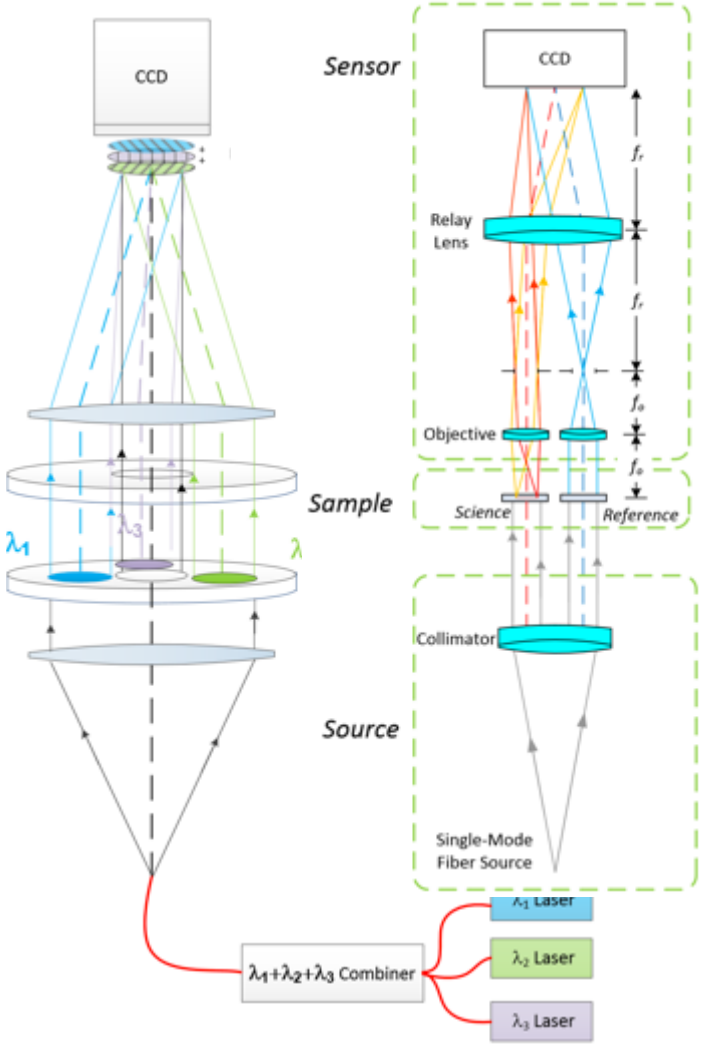


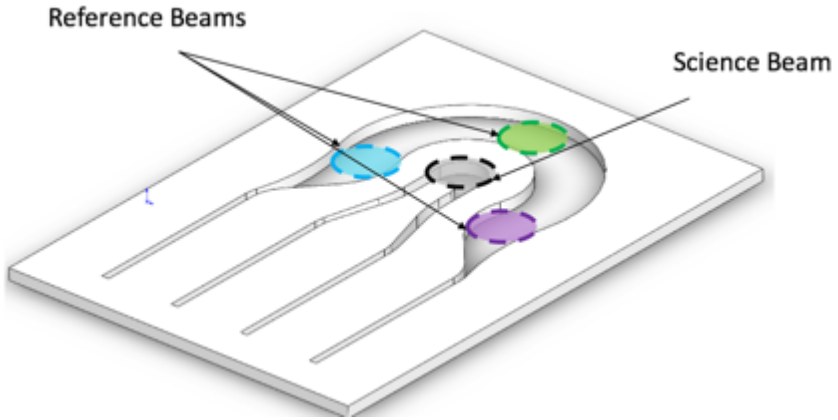
What can we learn from color?

Multiwavelength Architecture

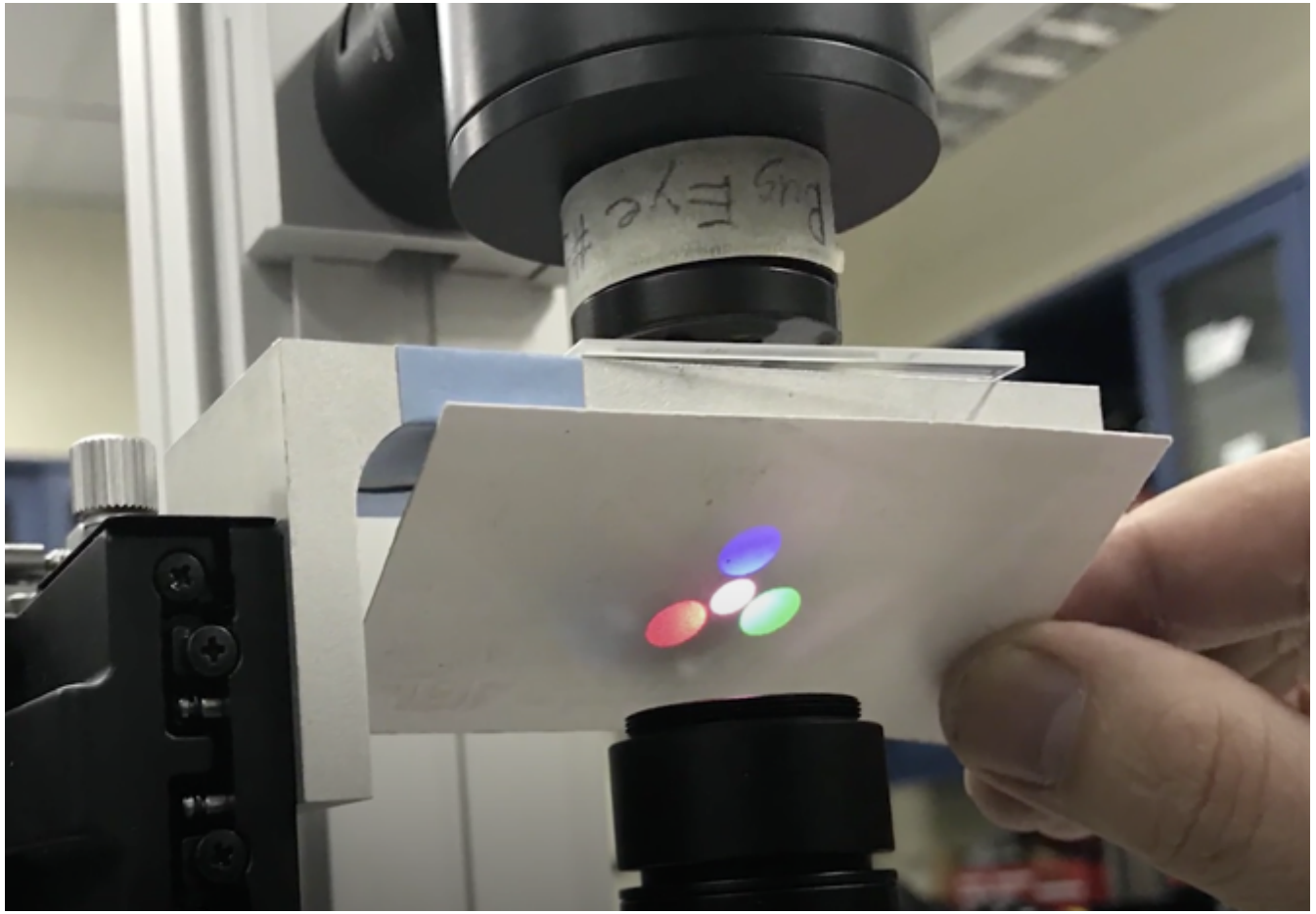




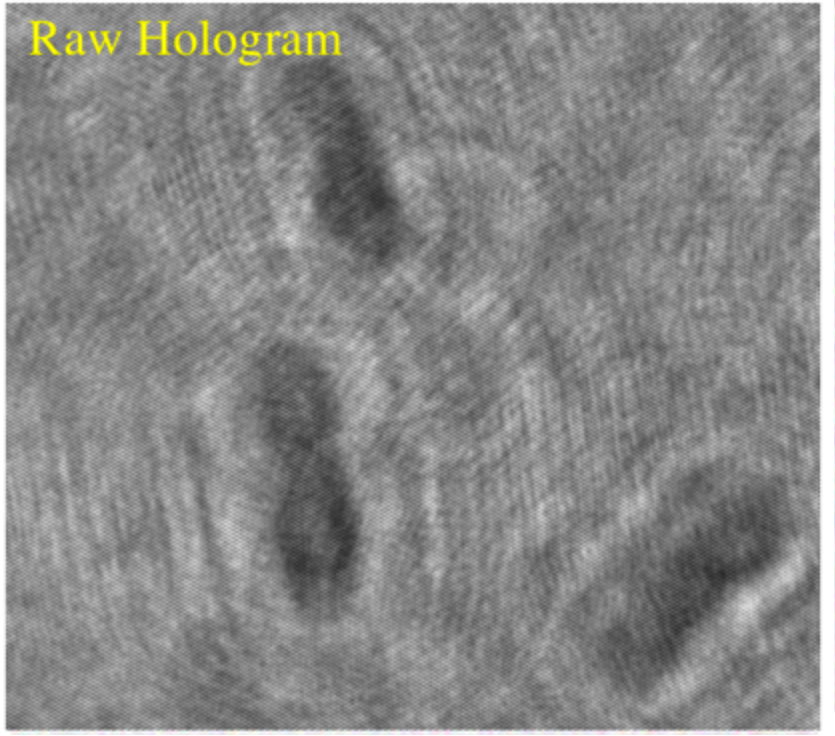




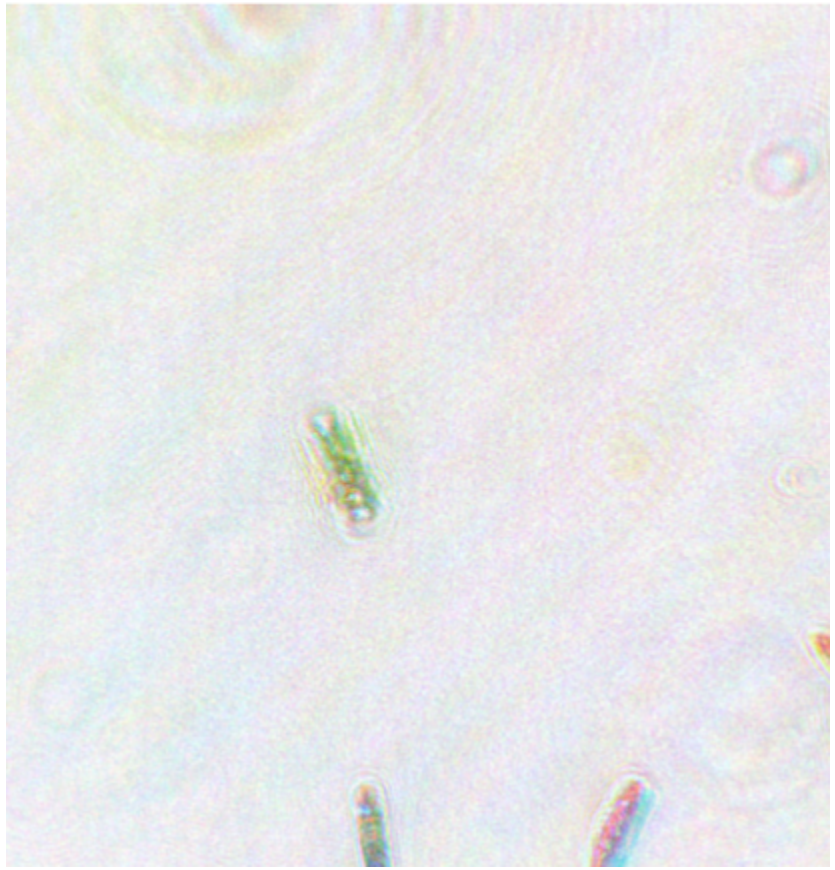
Multi-wavelength Hardware



Multiwavelength DHM Data



Multiwavelength DHM



Conclusion

- Discriminating extant life can be tricky.
- Our new suite of instruments, digital holographic microscopes – or DHMs, provide extra capabilities
- In this talk we have introduced a new discrimination method for DHM – multiwavelength (or color) sensing.
- This new method is a slight modification to our previous, common-mode instrument, so we lose no capabilities over the monochromatic design.
- It allows us another means of identifying bacterial species as well as intercellular structures.